TECHNIQUES FOR THE DISSECTION AND MOUNTING OF THE MALE (AEDEAGUS) AND FEMALE (SPERMATHECA) GENITALIA OF THE CHRYSOMELIDAE (COLEOPTERA)

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Abstract

Techniques for the dissection and mounting of the aedeagus and spermatheca of Chrysomelidae are described in detail. The major advantage of these techniques is the relatively short preparation time required per specimen, about 10 minutes for a male or about 15-20 minutes for a female. Techniques for the elimination of air bubbles within the genitalia are given. The appendix lists solutions/reagents, supplies needed and several sources, and techniques for making dissecting tools.

There is an increasing reliance on the characters provided by the male and female genitalia in the classification and determination of many groups of chrysomelid beetles. The method described by Arnett (1947) for male genitalia and that suggested by G. A. Samuelson (pers. comm.) for the spermatheca are general guides whose specific steps must be added by trial and error. I have greatly modified and refined these procedures into techniques whose major advantage is the relatively short preparation time required per specimen for routine dissection and mounting (male, about 10 minutes; female, about 15-20 minutes).

Although I have used these techniques primarily for the flea beetles (Alticinae), I have also used them for the examination of the genitalia in most other chrysomelid subfamilies and for some other beetle families. These techniques have been used for beetles primarily in the size range of 1-13 mm in length, but mostly in the 2-6 mm range where the aedeagus may be as short as about 0.7 mm and the spermatheca about 0.25 mm long.

Powell (1941) found that in the North American Chrysomelidae (subfamilies Alticinae, Lamprosominae, Megascelinae, and Sagrinae were not studied), the male genitalia are heavily chitinized. I can add that in the Alticinae, both the aedeagus and the spermatheca are usually heavily sclerotized. There is no apparent distortion in morphology when the techniques described below are used.

Terminology and Morphology

I use the term aedeagus to mean both the median lobe and the tegmen, as defined by Sharp and Muir (1912); see Figures 1a and 1b. The major reference on male genitalia for the chrysomelids of North America is the one by Powell (1941) cited above. These are the two basic references for male genitalia, and both have good bibliographies.

There is apparently no comparative or comprehensive work for the spermatheca of North American chrysomelids, a structure not included by Tan-

ner (1927) in his studies of female beetle genitalia. However, Spett and Lewitt (1926) give a fairly representative survey of European species. Labeled figures and brief descriptions of morphology are given by Lindroth and Palmén (1970) and Samuelson (1966, 1973). The basic morphological features are indicated in Figure 2.

Determination of Sex

The sex of almost all flea beetles and many other chrysomelids can be determined by examination of the 5th visible abdominal sternite (apical ventral plate). In males (Figure 3), paired indentations on the apical margin delimit a median lobe (usually concave) and the apical margin is surrounded by the apical margin of the pygidium (apical tergite or dorsal plate). In females (Figure 4), the apical margin is evenly rounded or smooth and it is not surrounded by the pygidium.

Dissection Technique

1. After removing the labels, soften the beetle by placing the mounted specimen in a 30 ml beaker of distilled water heated to just below the boiling point, for about 2 minutes (beetles longer than 4 mm require more time).
2. Transfer the beetle to a drop of distilled water in an elliptically-grooved depression slide using a pair of watchmaker's forceps. Remove pinned specimens from their pin. Pointed specimens are not much more difficult to work with than unmounted specimens.
3. While holding the beetle (ventral side up) with watchmaker's forceps, insert a hooked minuten-pin dissecting needle between the metacoxae and the first visible abdominal segment, sever the connecting membrane, and gently separate the abdomen.
4. Place the abdomen in a beaker of warm to hot, but not boiling, concentrated KOH for about 2 minutes in a 30 ml beaker, using 2 glass boiling beads; keep liquid above 7.5 ml by adding distilled water as required from a wash bottle.
5. Transfer the abdomen back to the water in the depression slide dorsum up; while holding the basal 2-3 sternites along the left margin with watchmaker's forceps, slit the left tergal margin from apex to base with a minuten-pin scalpel.
6. Return the abdomen to the KOH for another 2-3 minutes and then, if the abdominal tissue is not fairly well dissolved, briefly return the abdomen to the KOH for another minute or two as required.
7. Place the abdomen in acid alcohol for 1-2 minutes.
8. Put the abdomen into the appropriate rinse as follows:
   a) For *males*, put the abdomen into a 95% ETOH rinse for 1-2 minutes.
   b) For *females*, if it is a routine dissection for identification, put the abdomen into a 95% ETOH rinse for 1-2 minutes OR if the spermatheca is to be slide-mounted in Hoyer's for detailed study, transfer the abdomen from acid alcohol to a distilled water rinse for 1-2 minutes.
9. While in the appropriate rinse, separate the aedeagus or spermatheca from the tissue residue and dissect it out, using watchmaker's forceps and a hooked minuten-pin dissecting needle.
10. From the rinse, transfer the aedeagus or spermatheca to the appropriate medium for mounting as follows:
    a) Aedeagus: if less than 2 mm long, from a type-specimen, and/or to be studied in detail, transfer to glycerine; OR if longer than 2 mm and it is a routine identification, go directly through the final dry mount procedure. For details, see below under "Mounting."

b) Spermatheca: either put directly through the final mounting procedure for routine identification or through the Hoyer's procedure followed by the final mounting procedure if it is to be studied in detail. For details, see below under "Mounting."

11. After the aedeagus or spermatheca is transferred from the rinse watch glass, use watchmaker's forceps to transfer the abdomen to a small piece of paper towel or filter paper and immediately remount it. For details, see below under "Mounting."

Transfers and Solutions

All transfers of the aedeagus alone are done with a hooked minuten-pin dissecting needle.

Except for the final transfer, transfers of the spermatheca alone are done with a disposable pipette (without bulb) as follows: 1) cover the basal opening of pipette with a slightly moistened index finger, 2) lower the pipette tip into the solution next to the spermatheca, 3) release the index finger to permit capillary action to draw the spermatheca into the pipette, and 4) immerse the tip of pipette into the next solution and use a very gentle puff of air on the basal end to complete the transfer.

If the spermatheca sticks in the pipette, immediately add a couple of drops of the same solution to the basal end; if this does not wash it out, wait until the spermatheca comes free from the glass and gently puff again. For the final transfer to slide or card, use a hooked minuten-pin dissecting needle.

For each of the above solutions and the others mentioned below, use about 3-4 drops of solution per appropriately labeled Plant Quarantine watch glass, and keep it covered with another such watch glass. For additional details, see appendix under "Solutions."

Air Bubble Problems

To prevent the formation of air bubbles within the aedeagus or spermatheca, make the transfer into each new solution or medium quickly with complete submergence. When the aedeagus is totally submerged, the basal foramen (ventral opening) should be oriented toward the solution's surface. If air bubbles form inside the aedeagus, there are several possible ways of eliminating them: 1) while maintaining the above orientation, leave the aedeagus under illumination for several minutes to gently warm it; 2) return the aedeagus to the previous solution (acid alcohol is particularly effective); and/or 3) while maintaining the orientation, try to force the bubble out through the basal foramen by applying very gentle pressure to the ventral surface between the apex and bubble but close to the bubble, using the side (not the point!) of the minuten-pin dissecting needle. Occasionally a bubble may form inside the internal sac that cannot be expelled by the above technique; try to force the bubble out through the apical/median orifice by applying pressure between the basal foramen and the bubble but close to the bubble.

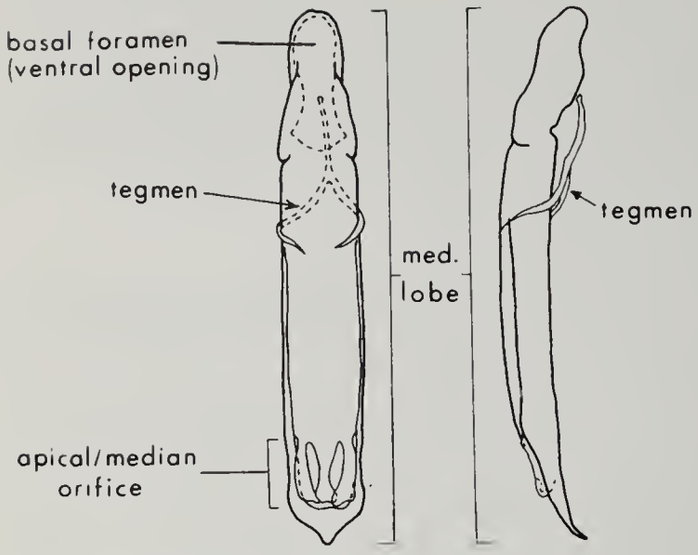
Rarely, an air bubble may form within the spermatheca. If it does, try the following, singly or in combination: 1) use the illuminator to warm the solution; 2) backtrack to previous solutions; and/or 3) as a last resort, apply very gentle pressure between the pump end and the bubble using the side of the minuten-pin dissecting needle.

Mounting

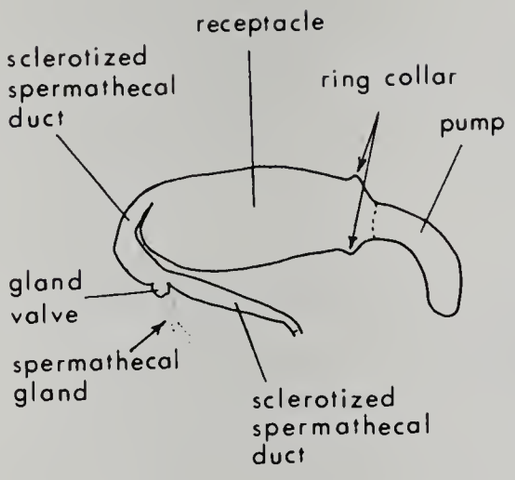
Abdomen. The way in which the abdomen is mounted is determined by how the beetle is mounted. The preparation is done as follows:

1. If the beetle is mounted on a point, place the abdomen, ventral surface up, in a small drop of LePage's water soluble glue on the point next to the specimen, oriented parallel to the longitudinal axis of the beetle and with the apex pointing posteriorly (Figure 5).

2. If the beetle is mounted on a card, place the abdomen, ventral surface up, in a small drop of LePage's glue on the card between the remounted specimen and the pin, oriented in the same axis as the beetle and with the apex pointing toward the pin.



1a 1b



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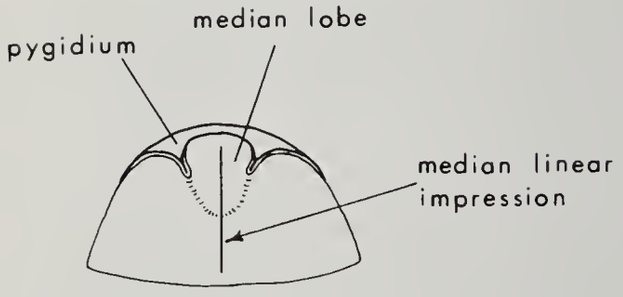

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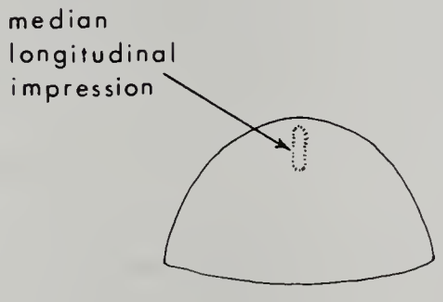

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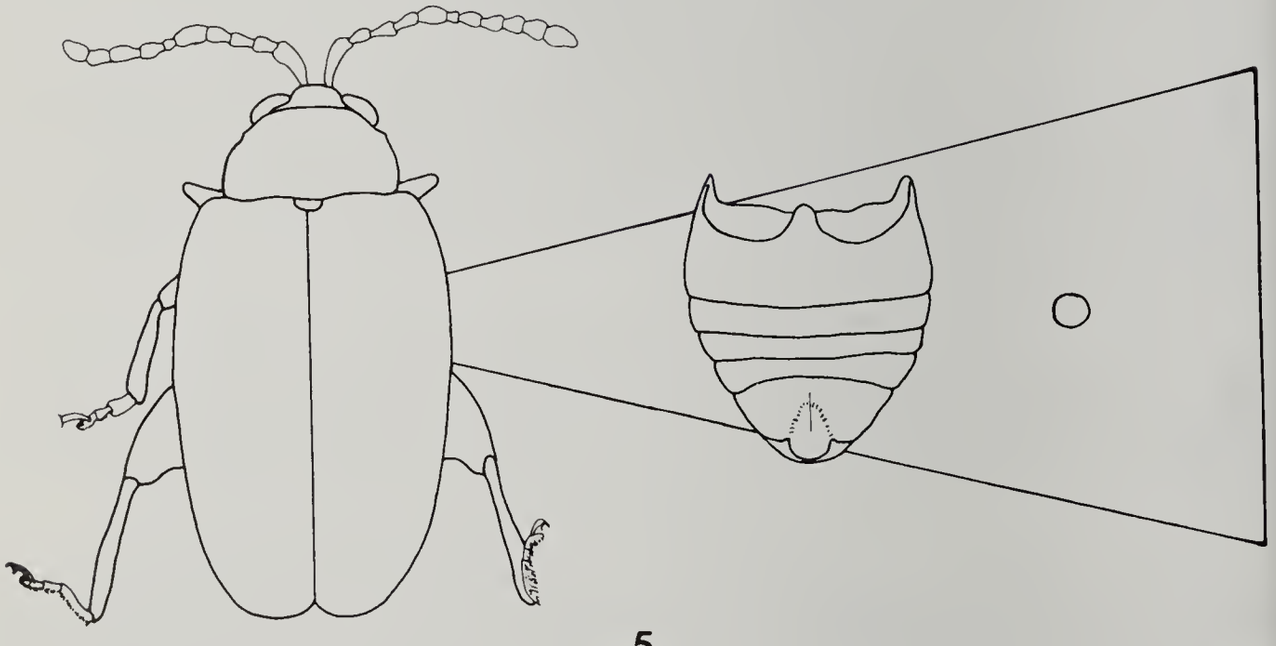

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Fig. 1, aedeagus: a) dorsal view, b) lateral view; Fig. 2, spermatheca; Fig. 3, male 5th abdominal sternite; Fig. 4, female 5th abdominal sternite; Fig. 5, mounting of abdomen on point.

3. If the beetle is pinned, mount the abdomen as described for pointed specimens (Figure 5) but on a small point which has already been pinned beneath the specimen. For details, see below under "Aedeagus, about 2 mm or longer."

Aedeagus, less than 2 mm long or from type-specimens. If the aedeagus is from a type-specimen and/or is less than 2 mm long, preparation is as follows:

1. Transfer it from the 95% ETOH rinse to glycerine for 1-2 minutes, and study.
2. After study, transfer it to a small size polyethylene genitalia vial (for type, use large size vial if appropriate) using a minuten-pin dissecting needle.
3. Add the stopper by inserting it along with a #2 insect pin (between the vial and stopper) to release any air pressure, then withdraw the pin while holding stopper in place by thumb.
4. Pin the vial through stopper and position it below the specimen's repinned data labels.

Aedeagus, about 2 mm or longer. If the aedeagus is about 2 mm or longer and to be studied, transfer it from the 95% ETOH rinse to glycerine. After study, return it to a 95% ETOH rinse for 2-5 minutes or longer if necessary. However, if the dissection is for routine identification, mount the aedeagus dry, directly from the 95% ETOH rinse.

The dry mount is made on the edge of a short mounting point (about 5 mm long) already positioned below the corresponding specimen on the pin. If the beetle is pointed, this second point should be shorter than the point used for mounting the beetle. If the specimen is pinned, the point should point toward the beetle's head and its tip should not extend beyond the beetle's head.

The mounting (Figures 6a, 6b) is done as follows:

1. Using an insect pin, put a small amount of LePage's water soluble glue on the edge of the point in the appropriate location.
2. Remove the aedeagus from the rinse using a minuten-pin dissecting needle.
3. Mount the aedeagus on the side of the point so that: a) it is attached or glued along its lateral edge for the basal 1/2 of its length, b) it is oriented in the same plane as the point but approximately parallel to the point's longitudinal axis, c) the apex of the aedeagus does not quite extend to the tip of the point.
4. With a wet fine camel's hair brush, lightly touch the junction between the aedeagus and glue both above and below to insure adherence.
5. Repin the specimen's data labels below this second point.

This mount provides maximum protection for the aedeagus and permits one to examine the entire apex as well as the dorsal, ventral and lateral surfaces without the problems presented by a genitalia vial mount.

Spermatheca, temporary mount in Hoyer's. If the spermatheca is to be studied in detail and/or illustrated, the preparation is as follows:

1. Transfer it to diluted Hoyer's mounting medium (2 drops Hoyer's/4 drops distilled water, in a Plant Quarantine watch glass).
2. Allow the solution to evaporate at room temperature, shielded from dust, until the consistency is that used for slide mounting (4-6 hours, less if a slide drying tray is used); if the Hoyer's becomes too thick and/or the pump collapses, add a drop or two of distilled water and repeat (if the pump collapses again, add 2-3 drops of water, and if a slide drying tray was used, reduce the temperature or evaporate at room temperature).
3. Mount it in Hoyer's and allow slide to dry for 5 days on a slide drying tray (about 45°C).
4. After study and/or illustration, soak the slide in distilled water to dissolve the Hoyer's.
5. Rinse the spermatheca in distilled water for 2-5 minutes.
6. Transfer it to 95% ETOH for 2-5 minutes.

Spermatheca, final mount.

1. Transfer the spermatheca from 95% ETOH to carbo-xylene (or to 100% ETOH)

for 1-2 minutes, and then to xylene for about one minute. If the pump collapses, return the spermatheca to 95% ETOH and repeat the procedure but leave it in the ETOH and carbo-xylene for a longer time; rarely will it need to be returned to distilled water or acid alcohol.

2. Transfer it with a minuten-pin dissecting needle to a slightly diluted drop of Turtox's Permamount mounting medium on a 3 × 7 mm paper card (for pointed specimens; otherwise, use a proportionately larger card) which has previously been sealed for 2/3 its length with diluted clear nail polish.

3. Position as in Figure 7, with the posterior or pump end towards the unsealed part of the card. During positioning, add additional xylene as needed with a fine camel's hair brush.

4. Add a small rectangle of cover glass (about 2.5 × 3-4 mm for pointed specimens; otherwise, use a proportionately larger rectangle) using watchmaker's forceps. Cut these in advance from rectangular cover slips with a diamond pencil.

5. Add additional Permamount around the edges of the cover slip.

6. Pin through the unsealed portion of the card (Figure 7) and position it below the specimen.

7. Repin the data labels below the card.

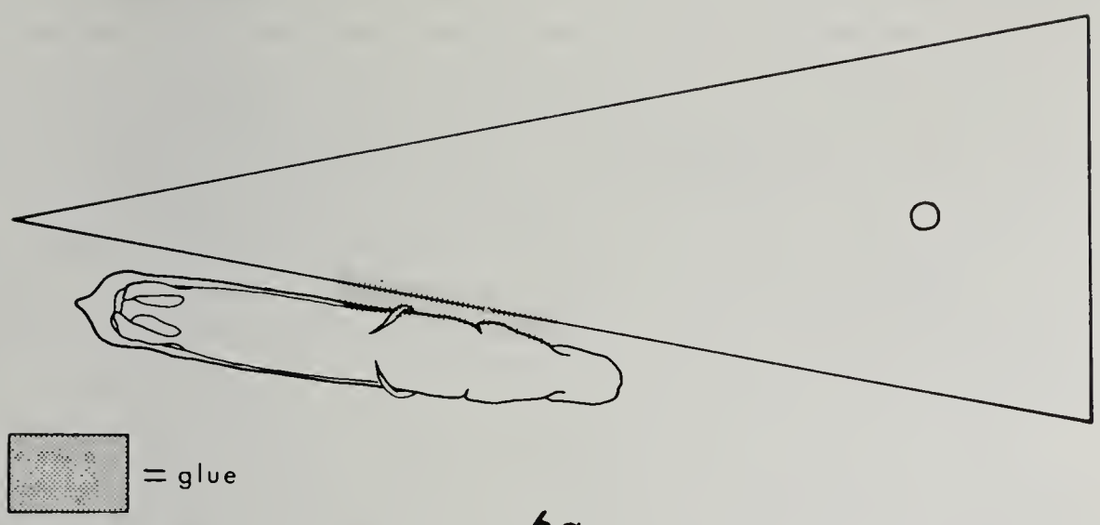
Suggestions and Modifications

Suggestions. There are two easy methods for markedly reducing the time required per specimen for preparation, and these 2 methods may be used separately or together. Both methods take advantage of preparing more than one specimen at the same time. However, with either method it is most efficient to prepare the genitalia of one sex at a time because of the varying complexities of, and the length of time required by, the different final mounting procedures. One method is to set up several series of the solutions/reagents described above. The other method is to treat the series of solutions/reagents as a production line. For example, when the abdomen in acid alcohol is transferred to its rinse, transfer the abdomen in KOH to the acid alcohol as soon as it is ready for the transfer, etc.

With both methods, each beetle is tagged by adding a differently numbered piece of paper to the pin. If using the multiple series method, be sure to mark each watch glass or beaker, not only to identify its contents, but also to indicate the correct beetle association. For example, I use "H-1" on the watch glass to indicate that its contents is Hoyer's medium and that the beetle associate is the specimen which I have tagged with the number one. When using the production line method, I prepare the beetles in numerical sequence as indicated by their tag number; this tells me that the first abdomen and genitalia ready for mounting belong to the specimen tagged with the number one, etc.

Modifications. When preparing the aedeagus or spermatheca of flea beetles or other beetles longer than 6 mm, the length of time required in each solution/reagent must be increased. In general, when the convection currents initiated by placement of the aedeagus or spermatheca into a new solution have stopped, fluid replacement is nearly complete. Therefore, run a beetle or two through these procedures and note the increased time required in the appropriate places. Now you have these techniques adjusted for the beetles of the size range in which you work.

For most males that are longer than about 20 mm, it may be more desirable to use and/or modify the dissection/extraction technique which was described by Arnett (1947) as follows: "After the specimen has been re-



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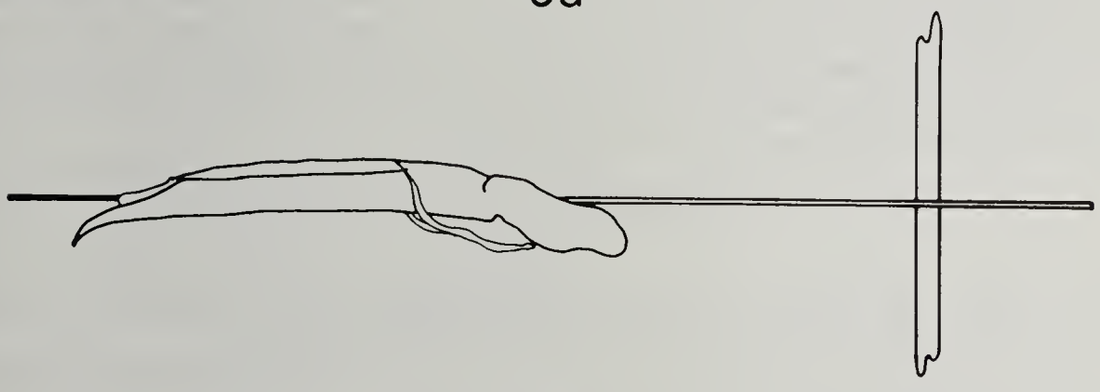

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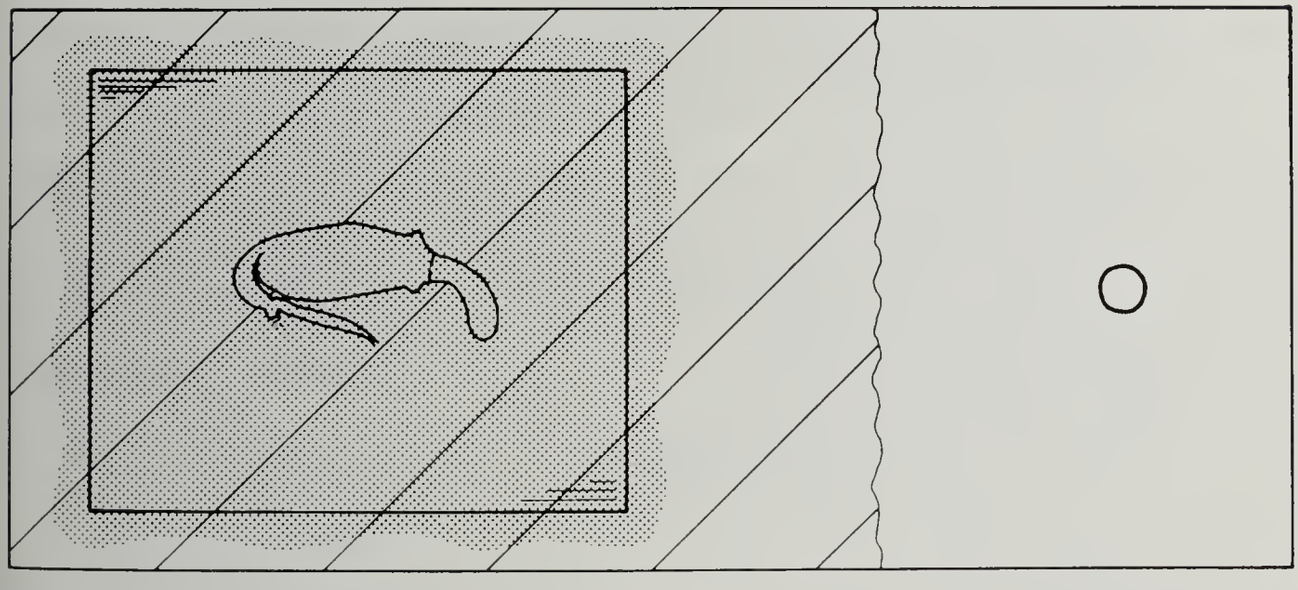

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Fig. 6, dry mount of aedeagus on point: a) dorsal view, b) lateral view; Fig. 7, final card mount of spermatheca.

laxed, the genitalia are removed by holding the specimen between the thumb and forefinger under low power of the binocular microscope and extracting the genitalia by means of a finely ground insect pin. Care must be taken not to cut or pierce the structures with the pin. If the specimen is thoroughly relaxed, the genitalia will easily pull out to a point where it may be cut off with fine scissors or pulled off with a pair of forceps." Arnett's method then is followed by the clearing and mounting techniques used above.

Each beetle group has its own peculiarities or problems which may necessitate modification of the techniques described here. For example, in some groups the aedeagus is so lightly sclerotized that staining is required to accentuate structures before study is possible.

Acknowledgments

I particularly thank Rupert Wenzel for critically reading the manuscript and offering many valuable suggestions, Ross H. Arnett, Jr. for teaching me the basic preparation technique for genitalia while I was a graduate student, and G. Allen Samuelson for sharing with me his method for the preparation of spermatheca of the Alticinae.

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Appendix

Solutions/Reagents

The stock solutions of the first 7 items listed below should be stored in dropper bottles. The remaining 8 are either stored in another kind of container or are mixed just prior to use in the appropriate glassware, as is indicated after their entry below.

1. Acid Alcohol (1 part glacial acetic acid to 3 parts of 70% ETOH)
2. Carbo-xylene (1 part dry carbolic acid to 1 part xylene)
3. Distilled water; also stored in wash bottle
4. 95% ETOH (ethyl alcohol); also stored in wash bottle
5. 100% ETOH
6. Ethyl Acetate
7. Xylene
8. Concentrated KOH: mix 5 pellets KOH/15 ml distilled water in 30 ml beaker

9. Diluted Hoyer's: mix 2 drops distilled water to 4 drops of Hoyer's in a Plant Quarantine watch glass
10. Diluted Clear Nail Polish: mix 3 parts clear nail polish to 1 part ethyl acetate, store in original bottle with brush
11. Elmer's Glue-All: store in original container
12. Permamount Mounting Medium: store in balsam bottle
13. LePage's water-soluble glue: store in balsam bottle
14. Glycerine: store in wide-mouth bottle
15. KOH pellets (potassium hydroxide): store in air-tight container

Supplies

The supplies listed below are arranged alphabetically. After each description, the suppliers (represented by capital letters) and their respective catalogue numbers (if available) are given. A list of selected suppliers follows this list of supplies.

If more than one supplier is listed, it means that each carries a comparable product. An asterisk indicates products I use; it indicates personal experience only, not endorsement.

Less expensive items can often be substituted for those listed below. However, I have found that those listed provide both long term dependability and ease of use.

Bead, glass boiling: Pyrex, 6 mm diameter.
E: #72-5406; H: #17W5008

Beaker, Pyrex glass: 30 ml (need 2)
E: #72-1205; F: 17-57-1; H: #17W4000

Bottle, balsam: wide mouth, Wheaton 400, with glass applicator rod and ground glass cap: size 45 ml (need 2)
E: #71-6700; F: #17-525

Bottle, glass dropper: Army type, Wheaton, flint glass, pipette ground in and rubber bulb: 4 oz (120 ml) (need 7)
E: #71-6466

Bottle, wash: Nalgene, one-piece, screw closure, polyethylene, 1,000 ml (need 2)
F: #17-566-6; H: #18W4078

Brush, #0000 Camel's hair: (need 2)
Art or drafting supply store

Cover slips, rectangular: 22 × 40 mm, thickness 1
A: #3316; E: #63-3121; H: #14W3127

Cover slips, round: size 1/2″ (12 mm), No. 0, thickness 1.
A: #3350; E: #63-3020

Forceps, pinning: (need 1)
H: #14W0241

Forceps, watchmaker's: stainless steel, Dumont Inox No. 5, 4 1/4″ long (need 2)
A: #6439; D: #4524; E: #62-4684; H: #14W1410; *Other Source

Gauze, wire: 4 × 4″ (need 1)
E: #70-6890

Glue, LePage's:
Discount, drug, grocery, hardware stores

Glue-All, Elmer's:
Discount, drug, grocery, hardware stores

Lamp, alcohol: Wheaton, with ground glass cap, size 4 oz. (need 1)
E: #70-6602; H: #17W0206

Minuten pin, stainless steel: 15 mm long, 0.20 mm in diameter
B; D: #1204S

Paper, card: 100% rag, linen ledger, long grain, 36 lbs.
Other source

Pencil, diamond point: sharp diamond chip mounted on metal in 6″ hexagonal aluminum handle (need 1)
*A: #6540

Pipette, glass disposable: (Pasteur pipettes) Pyrex, size 5 3/4″ long
A: #4640; E: #73-6060; F: #29-252-1: H: #17W1140

Slide, elliptically-grooved depression: micro culture slide, 76 × 26 mm, 5 mm thick, polished plate glass with polished oval concavity 44 mm long × 14 mm at widest portion × 2 mm deep (need 1)
G: #7048

Slide, one-end frosted microscope: size 25 × 75 mm, thickness 0.97 to 1.07 mm, one end frosted one side.
*A: #3050; E: #62-2010; H: #14W3508

Stick, wooden applicator: made of selected hardwood, round, 15.2 cm (6″) long
E: #70-6865; F: #75-846; *Other source

Tripod: iron ring 6 1/4″ outside diameter with copper alloy legs which must be cut to 4 1/4″ long (need 1)
E: #70-6972; H: #15W0730

Vial, polyethylene genitalia: small size 4.5 × 14 mm, large size 6 × 16 mm
C

Watch glass, Plant Quarantine: U. S. Bureau of Plant Industry model, similar to Syracuse, 27 mm outside dia., 20 mm inside dia. × 8 mm deep, beveled surface is ground matte (need 2 doz)
G: #9850

Suppliers

The suppliers listed below were selected because they sell to individuals, have low minimum-order amounts, and their catalogues are readily available. They are listed alphabetically and each has been assigned an upper-case reference letter.

A. Clay Adams, Div. of Becton, Dickinson & Co., Parsippany, NJ 07054, (201) 887-4800.

B. Clair Armin, 191 W. Palm Ave., Reedley, CA 93654, (209) 638-3729.

C. Arthropod Specialities Co., P.O. Box 1973, Sacramento, CA 95809.

D. BioQuip Products, P.O. Box 61, Santa Monica, CA 90406, (213) 322-6636.

E. Carolina Biological Supply Co.:
–Burlington, NC 27215, (919) 584-0381
–Powell Laboratories Division, Gladstone, OR 97027, (503) 656-1641.

F. MacMillan Science Co. (Turtox), 8200 South Hoyne Ave., Chicago, IL 60620, (312) 488-4100; 800-621-8980.

G. Arthur H. Thomas Co., Vine Street at Third, P.O. Box 779, Philadelphia, PA 19105, (215) 627-5600.

H. Ward's Natural Science Establishment, Inc.:
–P.O. Box 1712, Rochester, NY 14603, (716) 467-8400
–P.O. Box 1749, Monterey, CA 93940, (408) 375-7294.

Making dissecting tools

1. Soak 6″ long, round wooden applicator sticks overnight in a jar or beaker containing a 1″ depth of water.
2. Next day, using pinning forceps, insert the pointed or sharp end of a 15 mm long stainless steel minuten-pin into the center of the soaked end of the applicator stick to a depth of about 3-3.5 mm, and slowly and carefully remove it so as not to enlarge the hole.
3. Insert the blunt end of a minuten-pin into this hole (#2 above) using pinning forceps, and insert it to a depth of about 5 mm. It may be desirable to bend the minuten-pin (#5 below) and possibly, to also grind it to make a scalpel blade (#7 below) before insertion.

4. After drying the tool for 2-3 hours, add a drop of Elmer's Glue-All to the end of the stick at the base of the minuten-pin and let it dry overnight.
5. Next day, bend the apical 2.5 mm of the minuten-pin to the desired angle by grasping the pin with a pair of forceps on each side of point where the bend is to be made, and then bending with one pair of forceps. For good all-purpose bends, try bends of 35° to 45° from the longitudinal axis.
6. To make a hooked dissecting needle, bend the tip of the minuten-pin toward the stick with a pair of forceps.
7. To make a scalpel from a bent dissecting needle, grind the minuten-pin on each side from the bend to the tip, using a fine whet stone. The cutting edge should be formed on the inner edge. The minuten-pin's tip should also be ground sharp on the opposite edge. It is helpful to use a pair of forceps to hold the minuten-pin during the grinding process.

BOOK NOTICES

Western Forest Insects, by R. L. Furniss and V. M. Carolin. 1977. U. S. Dept. Agric. Misc. Publ. No. 1339, vii and 654 pp., illus. (Contains 172 pages on Coleoptera. Supersedes Insect Enemies of Western Forests, by F. P. Keen, 1938, revised 1952, USDA Misc. Publ. No. 273. Available from Government Printing Office, Washington, DC 20402, $8.75.)

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Simulation of ecological processes, by C. T. de Wit and J. Goudriaan. 1978. Halsted Press, Div. of John Wiley & Sons, Inc., 605 Third Ave., N.Y., NY 10016. Paper, 175 p., $15.95.